

SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response

Treatment with rituximab (RTX), a monoclonal antibody targeting CD20, constitutes an important therapeutic strategy for patients with inflammatory rheumatic diseases. Some recent reports have already highlighted the risk of SARS-CoV-2 infection in patients treated with RTX.¹⁻⁴ Besides the risk of a more severe disease course during B cell depleting therapy, a major concern relates to a risk of reduced immunogenicity of vaccination. Therefore, the question arises if patients should withhold or interrupt RTX therapy around COVID-19 vaccination or delay vaccination. To address this question, we have assessed antibody response and T cell mediated immune response to the BNT162b2 (Pfizer/BioNTech) vaccine in patients undergoing RTX treatment at the end of the treatment interval.

Five patients under regular and recent RTX treatment were selected for COVID-19 vaccination with BNT162b2 (Pfizer/BioNTech). A detailed description of the methods and the patient characteristics (online supplemental table S1) can be found in the online supplemental material. The last RTX infusion was administered between 4 and 12 months ago (online supplemental figure S1). At the time of the vaccination, peripheral CD19⁺ B cells could only be detected in two patients (online supplemental table S2). Antibodies against the SARS-CoV-2 nucleocapsid (NC) and the receptor-binding domain (RBD) of the spike protein were analysed 12–23 days following the second dose of BNT162b2. Sex-matched healthy individuals who had received two vaccinations with BNT162b2 (n=4) and unvaccinated healthy individuals (n=4) served as controls. No antibodies against the NC were detected in either group, implying no prior SARS-CoV-2 infection (data not shown). In three patients, no antibodies against the RBD were detected. Interestingly, in two patients with detectable CD19⁺ B cells, we determined a positive antibody response against the SARS-CoV-2 RBD, suggesting the development of a humoral immune response once peripheral B cells are repopulated (figure 1).

To determine a SARS-CoV-2 specific T cell reactivity, we measured interferon (IFN)- γ response to SARS-CoV-2 peptides in our patient cohort and control groups. All groups showed

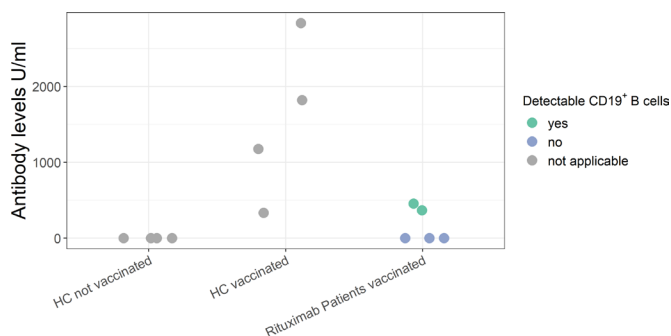


Figure 1 Humoral immune response in rituximab-treated patients. Antibodies to the receptor-binding domain (RBD) of the viral spike (S) protein was determined using an anti-SARS-CoV-2 S immunoassay. Rituximab-treated patients with detectable CD19⁺ peripheral B cells are labelled green. Patients with no peripheral B cells are labelled in blue. Vaccinated and not vaccinated healthy individuals served as a positive and negative control group. HC, healthy controls.

IFN- γ secretion on non-specific T cell stimulation of heparinised whole blood with mitogen. After stimulation with two different SARS-CoV-2 specific antigen mixes, IFN- γ response could be detected in the vaccinated healthy control group as well as in the patient cohort, independent of the humoral immune response (online supplemental figure S2). Of note, lower levels of IFN- γ were detected in one patient who concomitantly received intermediate prednisone dose.

In the current report, we could demonstrate that B cell depleting therapy with RTX affects the humoral immune response to SARS-CoV-2 vaccination in B cell depleted patients. However, humoral immune response was observed in patients who had measurable peripheral B cells following RTX treatment. These data are in line with very recent reports showing that RTX treatment might affect the antibody response to SARS-CoV-2 vaccination.^{5,6} However, we could here reveal a T cell mediated immune response even in B cell depleted patients. It will be important to understand if T cell immunity is important or possibly even sufficient to protect patients against infection with the virus on vaccination. Our data also indicate that RTX treatment may not have to preclude SARS-CoV-2 vaccination, since a cellular immune response will be mounted even in the absence of circulating B cells. Alternatively, in patients with stable disease delaying RTX treatment until after the second vaccination may be warranted and, therefore, vaccines with a short interval between first and second vaccination or those showing full protection after a single vaccination may be preferable. Importantly, in the presence of circulating B cells also a humoral immune response may be expected despite prior RTX therapy.

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Methods

Patients:

Patients followed routinely at our outpatient clinic were enrolled. A detailed patients characteristics can be found in **supplemental table S1**. Prior to SARS-CoV-2 vaccination, the treatment duration with Rituximab was on average 27 months, the mean number of Rituximab cycles administered per patient was 5.8. Blood was drawn during routine screening laboratory testing. Serum samples were stored at the Biobank of the Medical University of Vienna, a centralized facility for the preparation and storage of biomaterial with certified quality management (ISO 9001:2015)[1]. Ethical approval for this study was granted by the local ethics committee of the Medical University of Vienna, Austria. All control participants provided written informed consent to donate their samples for the evaluation of diagnostic tests in the course of a Healthy Donor Collection of the MedUni Wien Biobank (EK 404/2011).

Patient and public involvement:

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Anti-SARS-CoV-2 testing:

The Elecsys® Anti-SARS-CoV-2 S immunoassay was used for the quantitative determination of antibodies to the receptor-binding domain (RBD) of the viral spike (S) protein[2]. The quantitation range is between 0.4 and 2500.0 U/mL. Previous SARS-CoV-2 infection was ruled out by measuring nucleocapsid-specific antibodies with the qualitative Elecsys Anti-SARS-CoV-2 assay[3]. Both tests were performed on a cobas® e801 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Laboratory Medicine, Medical University of Vienna (certified acc. to ISO 9001:2015 and accredited acc. to ISO 15189:2012).

Assessment of SARS-CoV-2 specific T cell reactivity:

T cell reactivity to SARS-CoV-2 specific antigens was assessed by a commercially

available interferon- γ (IFN- γ)-release assay (QuantiFERON® SARS-CoV-2 RUO, Qiagen, Hilden, Germany). In brief, 1 mL of heparinized whole blood was incubated at a mean of 6h (range 4-7h) after blood collection at $37\pm 1^\circ\text{C}$ for 22 hours in each of four different tubes: a so called Mitogen tube containing a non-specific T cell stimulator served as a positive control; a Nil tube without any additives served as a negative control; two further tubes contained with different SARS-CoV-2-specific antigen mixes for stimulation of lymphocytes involved in cell-mediated immunity. After incubation, samples were centrifuged and plasma was stored at $<-70^\circ\text{C}$ until analysis. IFN- γ was subsequently quantified using a QuantiFERON® IFN- γ ELISA (Qiagen), applying an 8-point standard curve. Results were calculated from optical density values using the QuantiFERON® R&D software v5.3.0 (Qiagen). Results exceeding the standard curve were interpreted as >8.0 IU/mL. The IFN- γ levels obtained from the Mitogen and SARS-CoV-2 specific tubes were corrected for the background measured in Nil tubes. According to tuberculosis testing recommendations Nil-values <0.7 IU/mL and Mitogen - Nil values ≥ 0.5 IU/mL were applied as reference standards.

Supplemental Table S1: patient's characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
sex	female	male	male	female	female
Age (years)	28	71	73	45	49
diagnosis	IMNM	AAV	EGPA	SLE	MCTD
Major organ manifestations	Myositis	NSIP	Peripheral neuropathy, cardiac involvement, asthma	Arthritis, Tenosynovitis, Polyserositis	ILD (NSIP), Raynaud Syndrome, Esophageal motility disorder, Microstomia
Current disease specific therapies in addition to RTX	MMF 2G, Prednisone 10mg, Immunoglobulin therapy q4w	Prednisone 2.5mg	none	Methotrexate 5mg	MMF 2.5 G, Prednisone 2.5mg

SLE: Systemic Lupus Erythematosus; MCTD: Mixed Connective Tissue Disease, EGPA: Eosinophilic granulomatosis with polyangiitis, IMNM: Immune-Mediated Necrotizing Myopathy, AAV: antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, ILD: Interstitial Lung Disease, NSIP: Nonspecific interstitial Pneumonia, MMF: Mycophenolate Mofetil, q4w: every 4 weeks

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Hemoglobin g/dl (12-16)	11.0	14.1	12.9	13.8	13.4
Thrombocytes G/L (150-350)	213	197	187	160	376
Leukocytes G/L (4-10)	2.41	13.55	2.88	6.37	9.02
Lymphocytes %	15	18	20	33	16
CD3 ⁺ % (63 – 85)	52	98	80	91	92
CD3 ⁺ CD4 ⁺ % (31 – 64)	27	27	51	43	65
CD3 ⁺ CD8 ⁺ % (19 – 48)	23	70	27	49	27
CD3 ⁺ HLA-DR ⁺ % (3 – 17)	4	43	16	19	8
CD3 ⁺ 16&56 ⁺ % (5 - 27)	6	31	3	14	3
CD19 ⁺ %	<0.1	1	2	<0.1	<0.1
Monocytes %	7	7	9	6	11
Granulocytes %	78	75	71	61	72

Supplemental table S2: Laboratory variables

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Figure legends

Supplemental Figure 1: Timeline of measurement of SARS-CoV2- antibody and T cell response in patients treated with Rituximab.

Supplemental Figure 2: T cell mediated immune response in Rituximab treated patients.

T cell reactivity to SARS-CoV-2 specific antigens was assessed by an IFN- γ releasing assay. IFN- γ levels were measured after stimulation with unspecific Mitogen or two different SARS-CoV-2-specific antigen mixes (Ag1, Ag2). Rituximab treated patients with detectable antibodies against the receptor-binding domain of the viral spike protein are labeled in green. Patients with no detectable antibodies are labeled in blue. Vaccinated and not vaccinated healthy individuals served as a positive and negative control group.

